EFFECTS OF THYROXINE AND PROPYLTHIOURACIL TREATMENT OF YOUNG MALE SPRAGUE-DAWLEY RATS ON POTASSIUM-INDUCED CONTRACTION OF THORACIC AORTA

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by

Jon S. Hade

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An abstract of a Thesis by
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The problem. This study investigated the effects of thyroxine and propylthiouracil treatment of young male Sprague-Dawley rats on aortic ring contraction induced by increasing potassium concentrations.

Procedure. Three groups (N=6) of thirty-day-old male Sprague-Dawley rats were treated for nineteen days with thyroxine (200 micrograms by injection), propylthiouracil (via drinking water), or nothing. Starting on the nineteenth day, one rat from each group was sacrificed each day until all rats had been used. A section of aorta was excised and cleaned of its adventitia. A 2-3 millimeter ring was then cut from the section of aorta. The ring was mounted, placed in a ring bath, stretched to a one gram preload and allowed to incubate. At the end of the incubation period, increasing potassium concentrations were introduced into the indubation chamber in a serial order from low concentration to high concentration. Resulting contractions of the aortic rings due to the increasing potassium concentrations were recorded.

Findings. The results indicate that treatment with thyroxine and propylthiouracil affected the aortic ring contractility to increasing potassium concentrations. Although statistically significant difference was observed only at the highest concentration of potassium on the percent of maximal response measurement, alterations in contractile strength values were evident. The thyroxine treated group generated consistently lower tensions compared to the euthyroid controls, while the propylthiouracil treated group generated consistently higher tensions than the control group.

Conclusion. It can be determined from this study that treatment with thyroxine and propylthiouracil affected generated tension of thoracic aorta rings subjected to increasing potassium concentrations. Although the treatments did not result in significant differences of the contractile strengths, the consistent increases and decreases in generated ring tensions, caused by the treatments with propylthiouracil and thyroxine respectively, suggest that these treatments did affect potassium-induced contractions of the aortic rings.

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INTRODUCTION AND REVIEW OF LITERATURE

A study dealing with the effects of chemically induced hyperthyroidism and hypothyroidism on the contractile response of 2 mm rings cut from the thoracic aorta of rats was recently completed by Stratton (1985) at Drake University. The hyperthyroid rats were prepared by daily subcutaneous injections of 200 micrograms of L-thyroxine (TRX) over a two week period. Aortic rings cut from these rats showed significant decreases in contractile strength in concentration/response experiments with epinephrine (EPI) and norepinephrine (NE) when compared to euthyroid controls. The hypothyroid group was prepared by the administration of a 0.1% propylthiouracil (PTU) solution in their drinking water. These rings showed significant increases in contractility when compared to the euthyroid rats.

There are several possible explanations for these results. Gross anatomical changes in the vascular smooth muscle of the aorta which might have been induced by the hyperthyroid and hypothyroid states may have altered the contractility. An unpublished thesis done by Olszewski (1984) at Drake University compared histological cross sections of different vascular tissues, including the thoracic aorta, of TRX and PTU treated rats. No noticeable gross anatomical differences in vascular tissue were observed between any of the rat groups tested. However,

even though no gross anatomical changes were apparent, the possibility exists that some modifications may occur at the ultra-microscopic and cellular levels. Stratton (1985) compared the contractile strength of 2 mm rings cut from the thoracic aorta of control, TRX-treated and PTU-treated rat groups with and without pretreatment by the beta adrenoreceptor antagonist propranolol. Two rings were cut from each rat. One ring was incubated in a modified Krebs-Bicarbonate solution, the other ring was subjected to a 30 minute pretreatment with 10^{-6} M propranolol and then incubated in Krebs-Bicarbonate solution to prevent beta adrenoreceptor activity when stimulated by norepinephrine. Generated tension in the nonpretreated ring was significantly decreased in TRX-treated rats and significantly increased in PTU-treated rats compared to euthyroid controls. However, rings pretreated with propranolol showed increased contractile tensions when compared to those not pretreated, with the greatest increase in TRX-treated rats and the smallest increase in PTU-treated rats. Also, propranolol treated rings did not show the characteristic relaxation at high concentrations of NE as seen in rings not exposed to propranolol. This study suggests that the aortic contractility changes may be explained, in part, by increased beta adrenoreceptor activity in TRX-treated rats and decreased beta adrenoreceptor activity in PTU-treated rats.

Several recent studies have determined that the pathophysiological states of hyperthyroidism and hypothyroidism modify adrenoreceptor activity in several tissues. Alpha-adrenoreceptors and beta-2-adrenoreceptors are the primary receptors responsible for the activation of vascular smooth muscle. Beta-1-adrenoreceptors are primarily found in the heart and play little, if any, role in the activation of vascular smooth muscle. NE depolarizes vascular smooth muscle of the phasic and tonic. It has been shown that NE in higher than physiological concentrations increases the frequency of action potentials and induces depolarization in the rat aorta (Wahlstrom 1973). Vascular beta-2 stimulation causes vasodilation while alpha stimulation causes vasoconstriction. Vascular alpha adrenoreceptors have a greater affinity for NE than do vascular beta-2-adrenoreceptors. That is, vascular alpha adrenoreceptors are stimulated by low concentrations of norepinephrine, while considerably higher concentrations are needed to excite vascular beta-2-adrenoreceptors. At low NE concentrations an increase in tension will occur because of the high affinity of alpha receptors for NE at low concentrations. At high NE concentrations the alpha mediated contraction is reduced by the recruited stimulation of $beta_2$ receptors. Hyperthyroidism has been shown to increase the number of beta receptors in rat heart (Stiles and Lefkowitz 1982; Williams et al. 1977; Tsai and Chen

1978); and in human lymphocytes (Ginsberg et al. 1981). In hypothyroidism there appears to be decreased numbers of beta receptors in turkey erythrocytes (Bilezikian et al. 1979); in rat reticulocytes (Stiles et al. 1981); in rat heart (Banerjee and Kung 1977); and in rat cerebral cortex (Gross et al. 1980). In contrast to the increased and decreased numbers of beta receptors to hyperthyroidism and hypothyroidism respectively, changes in the number of alpha receptors opposite those of beta receptors may occur (Dillman 1983).

The actual cause for the increased number of beta receptors in response to hyperthyroidism is not known, but two possible mechanisms have been proposed by Williams et al. (1977): (1) the increase in beta receptor number in hyperthyroid heart may be caused by a thyroid hormone-induced augmentation of receptor synthesis; (2) the increase may be caused by the hormone-induced lowering of tissue and/or plasma catecholamine levels. Studies done on turkey erythrocytes (Bilezikian et al. 1979) suggest that thyroid hormone affects beta-receptor cyclic AMP interrelationship in turkey erythrocytes by two distinct mechanisms: (1) in hypothyroidism, both beta receptors and catecholamine-dependent cyclic AMP formation are coordinately decreased; (2) in hyperthyroidism beta receptors are unchanged, but there is an amplification of the hormonal signals so that occupation of a given number of receptors at physiological

concentrations of catecholamines tends to increase levels of AMP.

The numerous studies just described concerning hyperthyroidism and hypothyroidism make it apparent that these two thyroid states do effect the number of beta receptors on several tissues and organs. Further, they suggest that the observed changes in aortic contractility to NE in hyperthyroid and hypothyroid rats may be associated with changes in the number and/or affinity of beta receptors in the vascular smooth muscle cells of rat aorta.

This thesis will deal with the effects of hyperthyroidism and hypothyroidism on the contractile strength of rat aortic rings when stimulated with increasing concentrations of the nonspecific vasoconstrictor, potassium sulfate. It will examine changes in the contractile strength of 2 mm thoracic aorta rings from hyperthyroid and hypothyroid induced young male Sprague-Dawley rats when potassium sulfate is used in place of NE as the contractile agent. Potassium produces direct depolarization effects on vascular smooth muscle without the direct activation of adrenoreceptors. A lack of change in aortic ring contractility between treatment groups to increasing potassium concentrations would suggest that the changes may be due primarily to altered adrenoreceptor activity. However, changes in the contractile tension of the aortic rings to increasing potassium concentrations would suggest

that the two thyroid conditions cause changes in aortic vascular smooth muscle beyond those related exclusively to altered receptor activity.

Altering the potassium ion concentration around vascular smooth muscle cells produces changes in their state of contraction. Increased concentrations of K^+ in a bath causes some isolated blood vessels to contract (Altura et al. 1972; Bevan and Osher 1963). Many other vessels relax when the potassium concentration is raised to less than 15 mM, the aorta of the rat is such a vessel (Biamino and Wessel 1973). However, if the potassium concentration is elevated above 15-30 mM, contraction of the vessel usually results (Bohr and Goulet 1961).

One indirect and four direct mechanisms were suggested for the various actions of potassium on the vascular wall. The indirect mechanism involves the release of NE by sympathetic nerves. NE release can be promoted by reducing the extracellular concentration of potassium (Vanhoutte and Lorenz 1974), while NE release can be inhibited by slightly elevating the potassium ion concentration (Bonaccorsi et al. 1977a; Bonaccorsi et al. 1977b). The direct mechanisms of altered potassium on vascular smooth muscle are probably the result of one or more of the following: (1) alteration in membrane potential directly resulting from the changes in extracellular potassium concentrations; (2) alteration in cell membrane

permeability to sodium and potassium; (3) alteration in membrane potential caused by the electrogenic Na^+-K^+ transport system; and (4) alteration in intracellular sodium concentrations which, in turn, influence calcium transport (Sparks 1980). These mechanics are additive and so the effects of a particular change in the potassium concentration on a particular vascular smooth muscle depends on the relative importance of each of the mechanisms (Sparks 1980).

The aorta is composed of vascular smooth muscle cells which do not exhibit membrane potential spikes. Non-spiking vessels, as with spiking vessels, may exhibit relaxation when potassium concentration is slightly raised (Bonaccorsi et al. 1977a; Bohr and Goulet 1961). This is usually followed by a return in active tension toward the base line with a small increase or an actual contraction with higher concentrations. Electrical activity of the membrane seems to have the same pattern, suggesting that the transient relaxation can be linked to hyperpolarization of the membrane (Bonaccorsi et al. 1977a). Hyperpolarization may be due to stimulation of the electrogenic Na^+-K^+ active transport system. Some isolated blood vessels will only relax to potassium if they are pretreated with a low potassium concentration environment (Norton and Detar 1972). On the other hand the pump's activity is reduced in response to lowered extracellular potassium concentration

leading to a slight depolarization and contraction. The intracellular sodium concentration builds up in this case (Friedman et al. 1973). Relaxation then occurs with a slight increase in extracellular potassium concentration because the Na^+-K^+ pumps can be initiated without counteraction by a low intracellular sodium concentration (Sparks 1980).

The direct depolarization effect of increased extracellular potassium concentration seems to be in competition with several mechanisms that favor relaxation. First, in many vascular tissues, increased potassium concentrations stimulate the Na^+-K^+ electrogenic transport system, which can cause hyperpolarization. This seems to dominate tissues until the potassium concentration is above 10-15 mM. Secondly, elevated potassium concentration increases plasma membrane permeability to potassium (Sparks 1980). Thirdly, alteration in the intracellular sodium concentration that result from the increased extracellular potassium concentration may effect calcium exit and effect tension development (Sparks 1980). If the extracellular potassium concentration is raised above 20 mM, contraction of the vascular smooth muscle results. This is the point at which the depolarizing affect of increased potassium concentrations is greater than hyperpolarizing affects, and contraction results (Sparks 1980).

The increases and decreases in aortic contractile

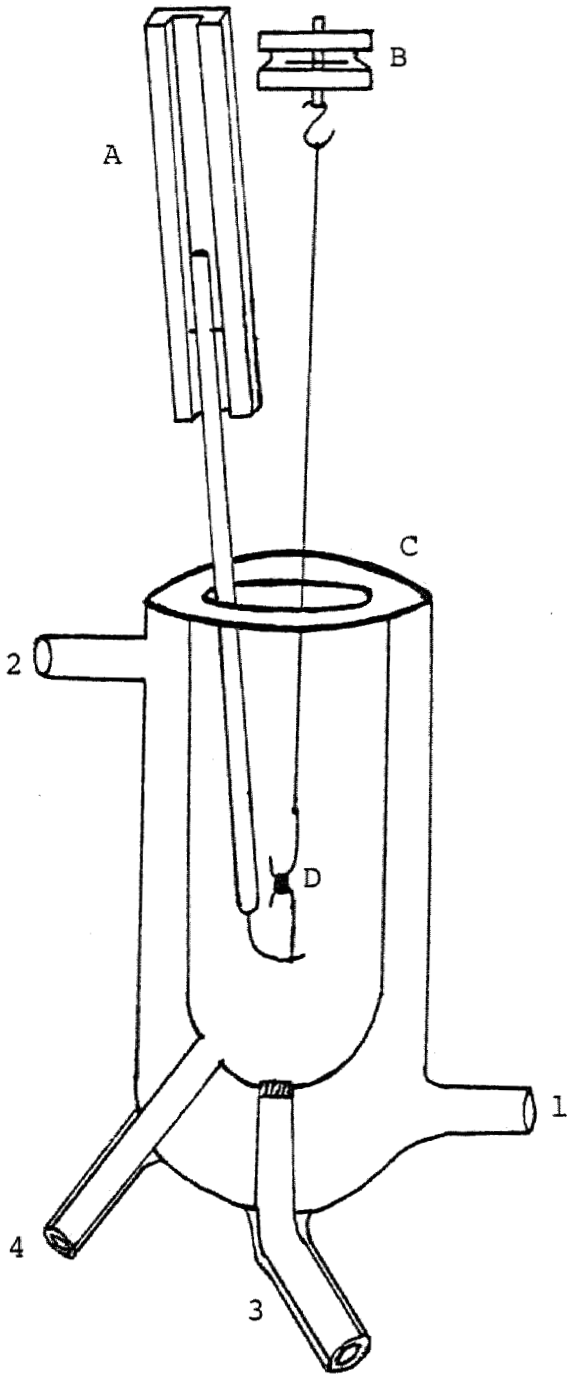
strength in hyperthyroid and hypothyroid rats respectively may be caused largely by increases and decreases in the number of adrenoreceptors or their affinity for receptor agonists. If the hypothesis is correct, there should be little, if any, change in the contractile strength of aortic rings, contracted by increased concentrations of potassium ions, from hyperthyroid and hypothyroid rats when compared to euthyroid controls. If, on the other hand, changes other than adrenoreceptor activity also occur, potassium concentration/response curves should be different when compared to controls.

MATERIALS AND METHODS

Experimental animals. Eighteen young male Sprague-Dawley rats were used, each weighing between 70-80 grams at the start of the experiment. All animals were housed three per cage under controlled temperature and lighting conditions. The rats were divided into three groups of six each. One group was given drinking water and Purina rat chow ad libitum and received daily 200 microgram subcutaneous injections of L-thyroxine (TRX) to render them hyperthyroid. Another group was rendered hypothyroid by the administration of a 0.1% propylthiouracil (PTU) solution in their drinking water. This group received both PTU water and Purina rat chow ad libitum. The last group (control rats) were also given drinking water and Purina rat chow ad libitum. Experimental rats were treated with TRX and PTU

for nineteen to twenty-four days before experimentation. Body weight of each rats was recorded throughout the treatment and experimental periods. Rats in a group were numbered one through six by a standard ear tag method. On the eighteenth day of treatment the metabolic rate of each rat was measured. Metabolic rates were calculated by indirect calorimetry in which oxygen consumption of each rat was measured. Oxygen consumption was multiplied by the caloric equivalent (.004825 calories/cc O_2 consumed) to determine the metabolic rate. Metabolic rates were used to help establish that the rats had been rendered hyperthyroid and hypothyroid by their given treatments. Hyperthyroid rats will generally show increased metabolic rates, while hypothyroid rats normally have lower metabolic rates when compared with euthyroid controls. Beginning on the nineteenth day of treatment rats numbered the same from each group were sacrificed. One rat from each of the three groups was sacrificed each day over a six-day period.

The Aortic ring apparatus (Figure 1). A 40 ml Radnoti muscle bath was used to incubate the aortic rings. 37°C water was circulated through the water jacket surrounding the chamber using a Precision Scientific stir pump. A 95% O_2/5% CO_2 gas mixture line was attached to the gas input nozzle of the muscle bath, and the bath drain was clamped off to prevent leakage of the incubation solution. The muscle bath was extended from a rigid support by means



A. Micro-manipulator
B. Grass force-displacement transducer
C. Radnoti muscle bath
   1. Water inflow
   2. Water outflow
   3. Air inflow
   4. Drain
D. Aortic ring

Figure 1. Ring Apparatus

of a jaw clamp. The clamp was attached to the support via a Harvard clamp which could be adjusted to raise or lower the muscle bath containing a modified Krebs-Bicarbonate solution (KBS) used for ring incubation. The millimolar composition of the KBS was: NaCl, 115; K_2SO_4, 50; NaH_2PO_4, 1.2; $NaHCO_3$, 25.0; CaCl, 1.7; glucose, 11.0; ethylenediamine tetraacetic acid (EDTA), 0.026. After aerating the KBS with 95% O_2/5% CO_2 gas mixture a Beckman pH meter (SS-2) was used to adjust the pH to 7.4.

Hooks used to suspend a ring in the bath preparation were assembled as follows: (1) the lower hook was anchored by polyethylene monofilament line to a bent dissection probe which was attached to a micro-manipulator used to adjust the preload of the rings, and (2) the upper hook was attached to a 13 centimeter gold chain which was linked, via the "S" hook, to a Grass force-displacement transducer (FT-03C; with no springs). Harvard clamps were used to secure both the micro-manipulator and the Grass force-displacement transducer to a rigid support.

Dissection and mounting of the aortic ring. Rats were sacrificed by a blow to the head. The thorax was then opened using a pair of surgical scissors. An incision was made 1-2 centimeters to the left of the sternum and extended from the abdominal region to the ventral and posterior aspect of the forelimb. Two mosquito hemostats were used to clamp a section of thoracic aorta, just rostral to the

diaphragm. One mosquito hemostat was then used to grasp and occlude the thoracic aorta just caudal to the aortic arch to minimize bleeding into the thoracic cavity. One more mosquito hemostat was then placed just caudal to the hemostat placed next to the aortic arch. A section of thoracic aorta was then cut free with a scalpal and pulled free from the body wall connective tissue by the mosquito hemostat. The excised section of aorta was rapidly placed in a shallow dissection dish which contained 37°C KBS which was continuously aerated with a 95% O_2/5% CO_2 gas mixture. The adventitia was carefully removed from the aorta section utilizing a pair of #5 microforceps (Hamilton Bell Suisse) and the section was cut into 2-3 millimeter rings using surgical scissors. Each aortic ring was mounted on the hooks and the muscle bath was raised so that the ring was suspended in the aerated, 37°C KBS. Each ring was mounted via two hooks which passed through the lumen and was then subjected to a preload tension of 1.0 gram and incubated for a period of 1.5 hours. The preload tension was constantly readjusted, using the micro-manipulator, to 1.0 gram during the incubation period.

At the end of the 1.5 hour equilibration period the normal KBS was drained out of the muscle bath via the chamber drain and replaced with a 30 millimolar (mM) potassium solution to initiate a precontraction of the ring. After 2-3 minutes, the 30 mM potassium solution was

drained from the muscle bath and the ring washed with normal KBS until the preload tension returned to 1.0 gram. Each ring was then serially contracted using eight different Krebs-Bicarbonate solutions with increasing concentrations of potassium (5, 10, 20, 30, 45, 60, 90, and 120 mM). When each potassium solution produced its maximal contraction, it was drained from the muscle bath and replaced by the next solution of increasing molarity. In this way, solutions of increasing potassium concentrations were used to obtain concentration/response tension curves for the aortic rings. The potassium solutions were held at 37°C in a Precision Scientific water bath (model 85) and aerated with a 95% O_2/5% CO_2 gas mixture before being introduced into the muscle bath. The increasing potassium concentrations were achieved by increasing the concentration of the K_2SO_4 in each solution. As the K_2SO_4 was increased the concentration of NaCl was decreased and the amount of glucose was increased in order to maintain a constant osmotic pressure (Price et al. 1981). Responses of each aortic ring to the increasing concentrations of potassium were measured with a Grass force-displacement transducer (FT-03C) whose output was monitored on a Beckman (Type RB) dynograph.

After stimulation of the ring with the 120 mM potassium solution, it was removed from the hooks by a pair of #5 microforceps (Hamilton Bell Suisse). The ring was put on #1 filter paper, blotted, and left to air dry for five

minutes. The wet ring weight was then measured on an analytical balance (Mettler).

During the ring incubation period, the thyroid gland of the rat was excised using surgical procedures explained by Waynforth (1980). The thyroid gland was placed on #1 filter paper, blotted dry, and weighed on an analytical balance (Mettler). The thyroid weights of each treatment group were compared to controls in order to further verify that the rats had been rendered hyperthyroid and hypothyroid by the TRX and PTU treatments respectively. Enlarged thyroid glands are characteristic of hypothyroid rats while undersized thyroid glands are seen in hyperthyroid rats.

Calibration of the force-displacement transducer. The force-displacement transducer was calibrated before the contractile strength of each aortic ring was measured. Each ring was suspended between the two hooks and placed in the KBS with no tension applied. The recording pen of the dynograph was then adjusted to the baseline using the balance control. This balanced out the weight of the chain, hooks, and unstretched ring. With the excitation switch "on," the sensitivity was increased to 50 microvolts. Next, a 2.0 gram weight was placed on the force-displacement transducer "S" hook (which was already connected to the 13 cm gold chain and the suspended unstretched aortic ring). The vernier sensitivity was adjusted to give a recording pen deflection of 5.0 centimeters (full scale

deflection). The 2.0 gram weight was then removed. If the recording pen did not return to the baseline, the balance control was readjusted. The 2.0 gram weight was then resuspended from the "S" hook. If the 2.0 gram weight did not produce the desired 5.0 centimeter deflection, the entire procedure was repeated. After proper calibration, the tension on the aortic ring was slowly increased by adjusting the micro-manipulator and stretching the ring until the recording pen gave a one-half scale deflection (2.5 cm). This deflection indicated that a 1.0 gram preload had been placed on the ring.

Calculations. The contractile tension of every ring at each potassium concentration was expressed as contractile force measured in milligrams (mg) divided by the weight of the ring also expressed in milligrams (mg). In addition, the contractile tension produced at each potassium concentration was divided by the maximal tension produced. This gave the percent of maximal tension produced by every ring at each potassium concentration. These two calculations normalize the tension of every ring so that the rings of each group might be compared to one another.

Statistics. Contractile response for each of the three groups of rats at the eight concentrations of potassium are presented as the mean plus or minus the standard error (S.E.). A two-tailed t-test for independent samples was used to compare means of the two experimental

groups to the control groups. $P<.05$ was considered significant.

Results for thyroid weights, metabolic rates, rat weights, and ring weights are presented as the mean plus or minus the standard error (S.E.). The two-tailed t-test for independent samples was used to compare means of the two experimental groups to the control group. $P<.05$ was considered significant.

The potassium concentration at which one-half the maximal response was produced (ED_{50}) was calculated for every ring. The standard EC_{50}'s are presented as the mean plus or minus the standard error. A two-tailed t-test for independent samples was used to compare means of the two experimental groups to the control group. $P<.05$ was considered significant.

RESULTS

Body weight. The mean body weights of both the PTU-treated and the TRX-treated groups were significantly lower ($p<.05$) than the control rats. The body weights are all expressed to the nearest gram. Table 1 shows the weight gain for each rat on every day of treatment and the overall mean weight gains of each group. The mean weight gains represent the difference between the starting weight and the weight on the eighteenth day of treatment. Figure 2 shows the overall mean weight gain of each group plus the standard error.

Table 1. Rat Weight Gain (grams)

Rat Number	Control	PTU	Thyroxine
1	150	112	136
2	133	111	95
3	157	111	103
4	126	98	122
5	125	113	107
6	137	129	117
Mean	138±4.8	112±3.7*	113±6.0*

*Significantly different from control ($p<.05$), (t-test).

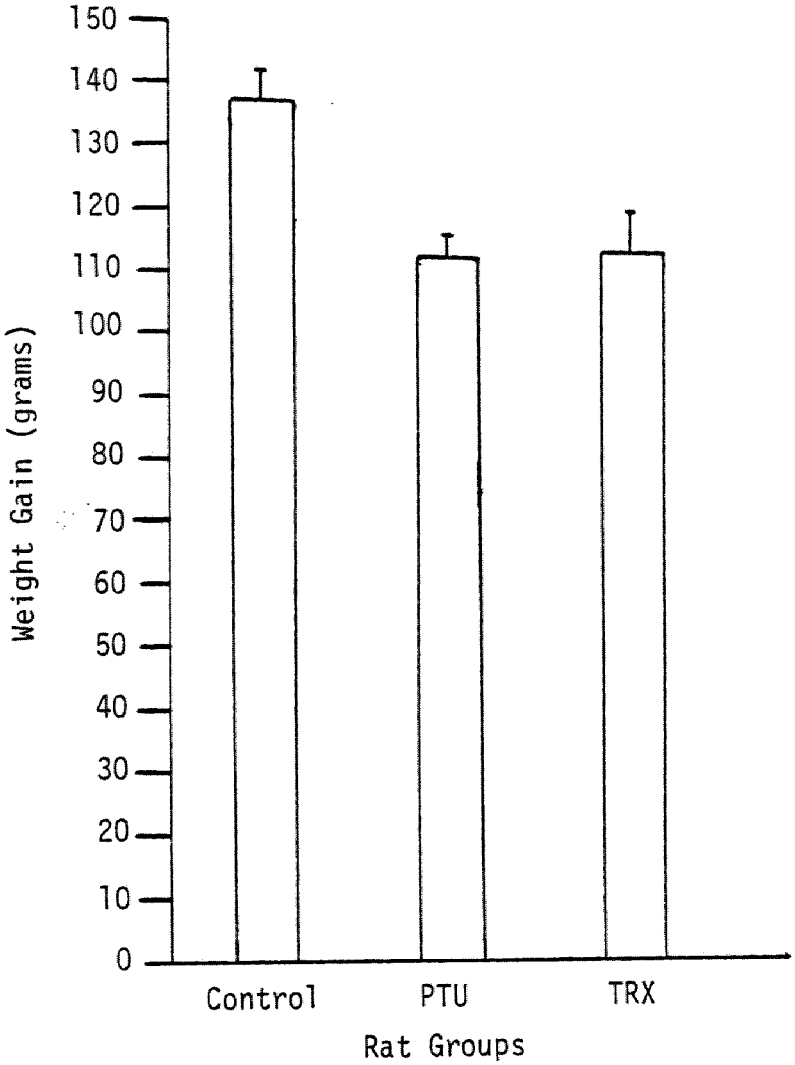


Figure 2. Mean Rat Weight Gains

Metabolic rate. The mean metabolic rate of the TRX group was significantly higher ($p<.05$) than the control group. The mean metabolic rate of the PTU group was significantly lower than the control group. The metabolic rates are expressed as calories/hour/meter^2. Table 2 shows the metabolic rate of each rat and the mean metabolic rate of each rat group. The metabolic rates were determined on the eighteenth day of treatment. Figure 3 shows the mean metabolic rate of each rat group plus the standard error.

Thyroid weights. The mean thyroid weights of the PTU rats were significantly larger ($p<.05$) than the control rats. However, there was no significant difference in mean thyroid weight between the control group and the TRX group, though the thyroid glands of the TRX group were consistently smaller than those of the control group. Table 3 shows the thyroid weight of each rat and the mean thyroid weight of each rat group. Figure 4 shows the mean thyroid weight for each group plus the standard error. Thyroid weights are expressed in milligrams (mg).

Ring weights. There was no significant difference in mean ring weights between any of the three groups of rats. Table 4 expresses the ring weight of each test rat and the mean ring weight of each group plus the standard error. Figure 5 shows the mean ring weight for each rat group plus the standard error. Ring weights are expressed in milligrams (mg).

Table 2. Metabolic Rate ($Cal/Hr/M^2$)

Rat Number	Control	PTU	Thyroxine
1	54.5	31.9	67.1
2	49.9	43.7	81.6
3	63.1	44.7	87.0
4	37.5	36.7	89.6
5	58.7	48.1	94.7
6	50.5	40.5	116.5
Mean	52.4±3.3	40.9±2.2*	89.4±6.7*

*Significantly different from control ($p=<.05$), (t-test).

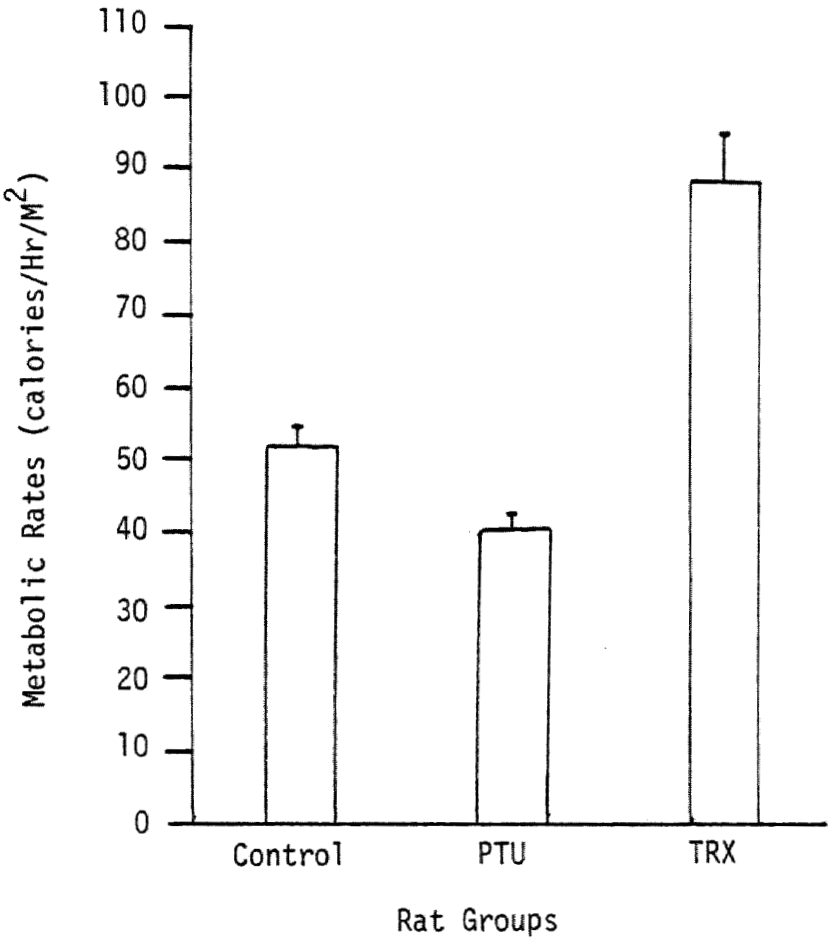


Figure 3. Mean Metabolic Rates

Table 3. Thyroid Weight (milligrams)

Rat Number	Control	PTU	Thyroxine
1	11.0	43.8	2.0
2	6.1	69.0	8.7
3	15.0	49.6	7.8
4	18.9	43.0	8.4
5	16.9	80.3	7.5
6	20.7	55.2	4.8
Mean	14.7±2.0	56.8±5.6*	6.5±1.1*

*Significantly different from control ($p=<.05$), (t-test).

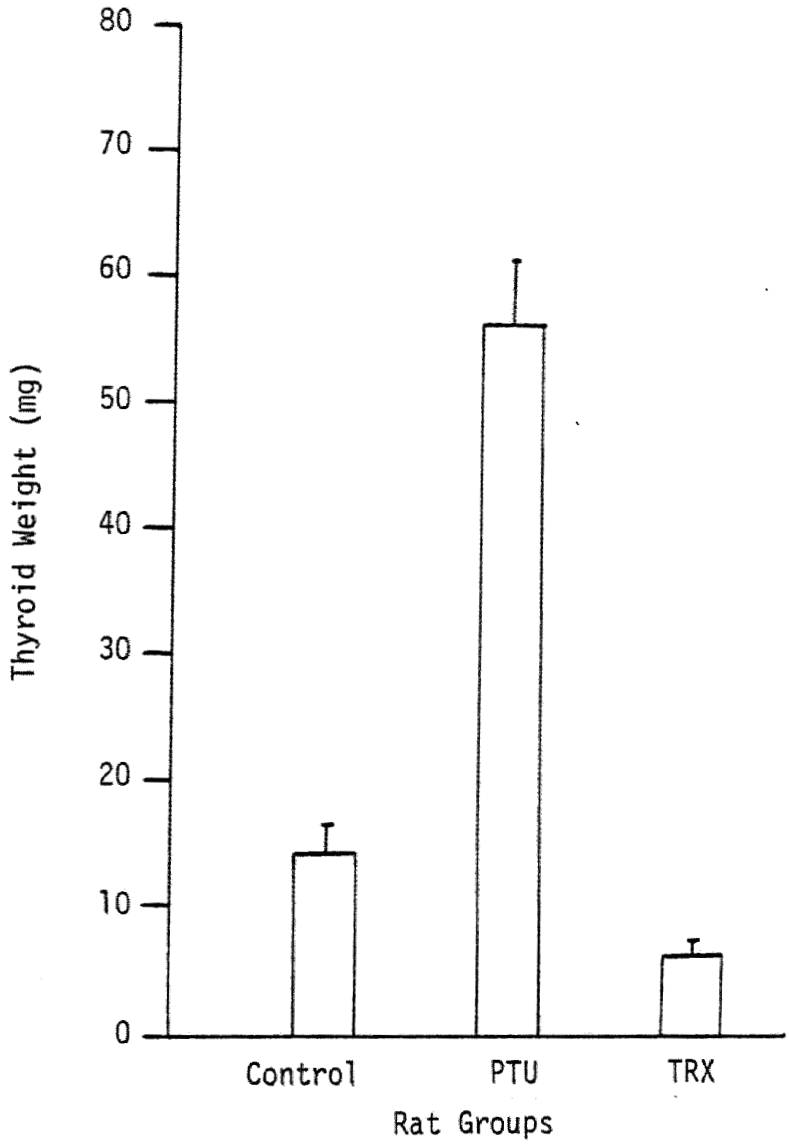


Figure 4. Mean Thyroid Weights.

Table 4. Thoracic Aorta Wet Ring Weight (milligrams)

Rat Number	Control	PTU	Thyroxine
1	1.4	1.2	1.1
2	1.5	1.4	0.9
3	1.1	1.3	1.6
4	0.9	0.8	2.1
5	2.0	2.2	1.3
6	0.9	1.8	1.0
Mean	1.3±0.2	1.5±0.2*	1.3±0.1*

*Significantly different from control ($p<.05$), (t-test).

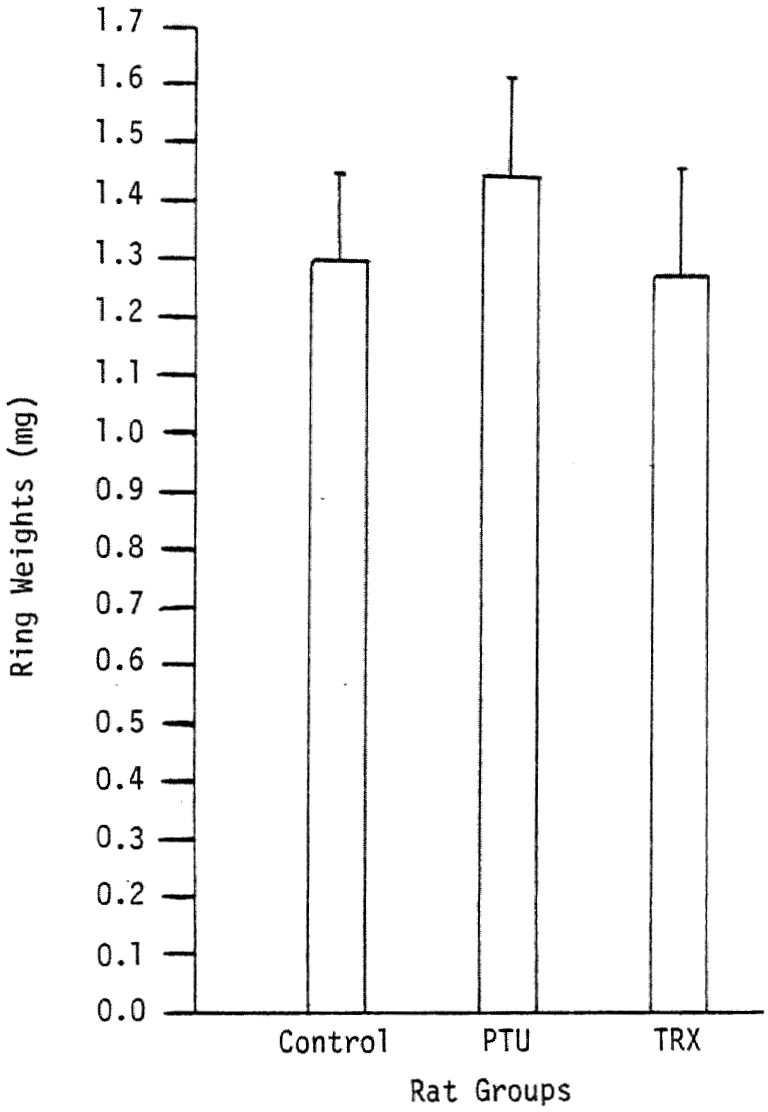


Figure 5. Mean Ring Weights.

Contractile force. The contractile force of each ring was expressed in two ways: (1) milligrams of tension/milligrams of wet ring weight, and (2) percent of maximal contraction. Tables 5 and 6 indicate the figures for these two measurements respectively for each ring as it was subjected to increasing concentrations of potassium ions. There were no significant differences between the generated contractile tension of any of the three rat groups with potassium concentrations up to 45 mM. However, above 45 mM the contractile force of the PTU-treated group was consistently higher than either the control group or the TRX-treated group. Nevertheless, there was a significant difference ($p<.05$) between the PTU-treated group and the TRX-treated group only at the highest potassium concentration of 120 mM for the percent maximal response. No significant difference was shown between any of the three groups of rats at any potassium concentration when the response was normalized to milligrams of tension/milligrams wet ring weight. The contractile forces for both milligrams of tension/milligrams wet ring weight and the percent of maximal response measurements are illustrated in Figures 6 and 7 respectively. The data points illustrate the mean contractile force of each group for each potassium concentration plus or minus the standard error.

EC_{50}'s. No significant difference in EC_{50}'s was shown between any of the three rat groups. The mean EC_{50}'s

Table 5. Mean Normalized Ring Tension (milligrams tension/milligrams wet ring weight)

Potassium Concentration (mM)	Control	PTU	Thyroxine
5	94.5±20.0	98.7±15.9	62.2±4.5
10	170.8±35.1	213.7±41.2	144.6±21.0
20	214.9±46.1	268.9±50.2	198.8±30.4
30	251.9±55.9	350.0±55.5	205.2±31.7
45	257.8±51.8	364.9±57.1	223.4±36.2
60	248.8±61.2	344.1±66.1	207.5±40.2
90	228.7±61.6	345.4±63.7	179.7±38.8
120	224.3±61.6	334.5±60.8	157.2±38.4

*Significantly different from control ($p<.05$), (t-test).

Table 6. Percent of Maximum Response

Potassium Concentration (mM)	Control	PTU	Thyroxine
5	37.5±5.3	28.4±4.0	30.4±4.8
10	65.2±6.1	56.5±3.8	64.6±3.3
20	78.2±5.7	83.8±4.5	87.6±1.6
30	91.8±4.9	94.3±2.1	89.9±2.9
45	95.0±2.5	98.5±1.1	97.4±1.6
60	85.7±8.2	92.5±1.3	88.0±7.9
90	76.0±9.2	94.3±1.2	74.8±8.6
120	74.3±8.6	91.8±1.6	56.8±9.3*

*Significantly different from control (p= .05), (t-test).

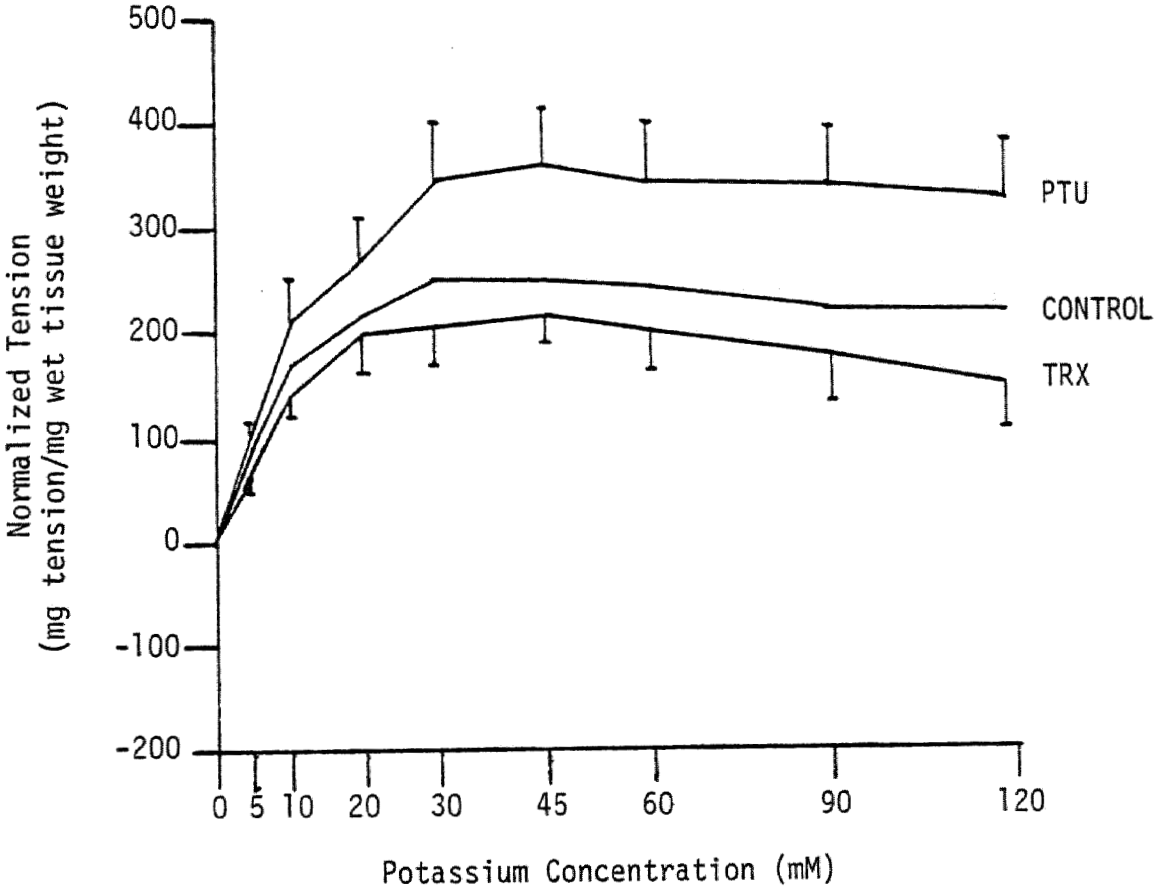


Figure 6. Aortic Ring Contractility (mean normalized ring tension).

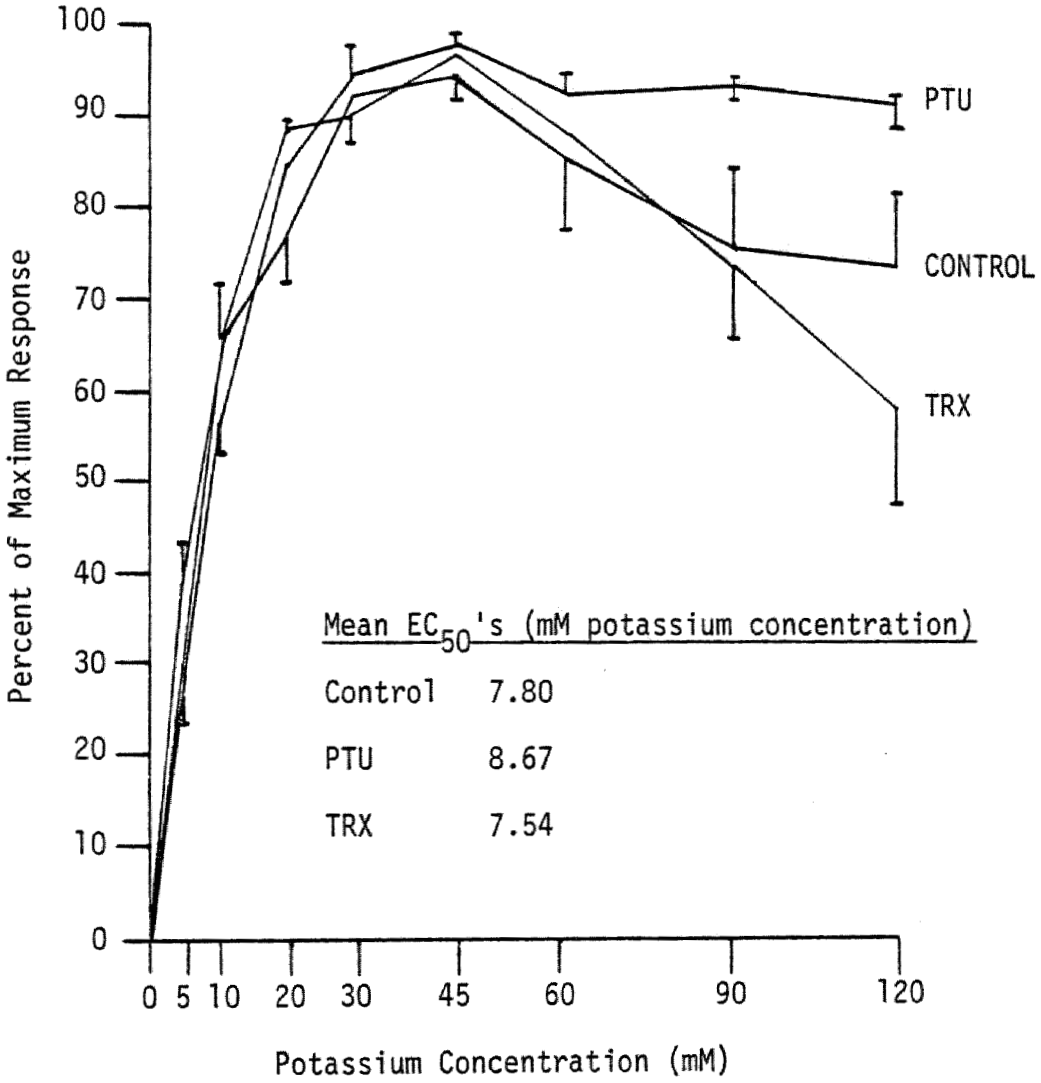


Figure 7. Aortic Ring Contractility (percent of maximum response.

are shown with Figure 7 (percent of maximum response). EC_{50}'s are expressed as mM potassium concentration.

DISCUSSION

Rats treated with PTU showed a significant decrease in both mean body weight gain and mean metabolic rate and showed a significant increase in mean thyroid weight when compared to the control group. Rats treated with TRX showed a significant decrease in mean body weight gain and a significant increase in the mean metabolic rate when compared to the control group. Thyroid weights in this group of rats were consistently less than controls but not significantly so. These results confirm that hyperthyroid and hypothyroid states were induced by treating the rats with TRX and PTU respectively.

Previous studies done by Stratton (1985) have demonstrated a decrease in maximal contractile force in aortic rings from TRX-treated rats and an increased contractile response in rings from PTU-treated rats when the rings were exposed to epinephrine and/or norepinephrine. These results are thought to be caused by increased beta and/or decreased alpha-adrenoreceptor activity in the TRX-induced hyperthyroid rats and decreased beta and/or increased alpha-adrenoreceptor activity in PTU-induced hypothyroid rats. In the present study, rings from PTU and TRX-treated rats were contracted in concentration/response experiments with increasing concentration of potassium which

caused contraction of vascular smooth muscle by depolarizing the membrane instead of directly activating receptor sites. If the findings of the study by Stratton (1985) were caused completely and exclusively by increases and decreases in adrenoreceptor activity in the aortae, no difference in responses of the aortae from different treatment groups would be expected when subjected to potassium induced contraction.

Nineteen to twenty-four days of treatment with TRX and PTU produced no significant differences in contractile tension values (mg tension/mg wet ring weight), however, the PTU group generated consistently higher contractile responses than did the control group while the TRX group generated consistently lower values. The PTU group also recorded higher percent maximal response values from the 45 mM through the 120 mM potassium concentration levels, though these values were not significantly different except at the highest potassium concentration. These differences indicate that pathophysiological changes other than those of adrenoreceptor activity in the vascular smooth muscle of the thoracic aorta may have been caused by the hyperthyroid and the hypothyroid conditions. Nevertheless, one could still make a case for the difference in contractile response being due to altered adrenoreceptor activity totally since norepinephrine is released by nerve endings in the vascular tissue when the concentration of extracellular K^+ ions is

increased (Vanhoutte and Lorenz 1974). Release of norepinephrine by the nerve endings would cause stimulation of both alpha and beta receptors. Stimulation of the beta receptors with norepinephrine causes a relaxation of the muscle, while stimulation of the alpha receptors with norepinephrine causes muscle contraction. If the number or activity of beta and/or alpha-adrenoreceptors were altered by the hyperthyroid and hypothyroid states, the release of norepinephrine by the nerve endings due to increased extracellular potassium ion concentrations could explain the differences in contractile force in the experimental groups. However, since rat aorta is only sparsely innervated, this possibility is probably not sufficient to explain the differences observed here. Therefore, it seems likely that other fundamental changes have occurred in the contractile capabilities of thoracic vascular smooth muscle in the two pathophysiological thyroid states. Even though no gross anatomical changes were observed in the thoracic aorta of the TRX- and PTU-treated rats when compared to the control groups (Olszewski 1984), such changes might still contribute to the changes in contractile response. The abnormal thyroid states might also produce significant changes in vascular smooth muscle biochemistry and metabolism which could contribute to the altered contractility.

Normalization of the contractile response values of

the rings was achieved by dividing the tension, measured in milligrams, produced by each ring by the wet weight of the ring which produced the tension. Since no significant differences were found in the mean ring weights between any of the three rat groups, it is reasonable to assume that the ring weights and the normalization procedure did not contribute to the observed altered contractile response.

The lack of statistical difference between contractile strengths of thoracic aortae in this study, together with the knowledge that adrenergic agonists cause significant differences in contractile responses in hyperthyroid and hypothyroid rats (Stratton 1985) make it reasonable to assume that the changes in contractile strength of thoracic aortae are caused at least in part by changing numbers or activity of adrenoreceptors in response to fluctuations in the amounts of circulating thyroid hormones. Yet, the fact that potassium-induced contractions were not essentially identical in the treatment groups suggests that changes in addition to those related to adrenoreceptors must have also occurred in response to the treatment with TRX and PTU. Changes in vascular tissue, other than those of adrenoreceptor activity have been discussed and may explain some of the contractile differences in the two pathophysiological thyroid states.

CONCLUSION

The influence of hyperthyroidism and hypothyroidism on the contractile strength of rat thoracic aorta was investigated using increasing potassium concentrations to initiate the contractile process of two millimeter aortic rings. Contractile responses were measured from aortic rings of euthyroid rats, rats made hyperthyroid by daily injections of L-thyroxine (200 micrograms), and rats rendered hypothyroid by adding propylthiouracil (0.1%) to their drinking water.

Percent maximum response and contractile strength, measured in milligrams tension/milligrams wet ring weight, were the two measurements determined from each ring. Results of the statistical examinations on the contractile strengths revealed that there was virtually no significant difference of thoracic aorta contractile strength between any of the three rat groups. Nevertheless, the aorta from TRX-induced hyperthyroid rats produced consistently less tension than euthyroid controls while aorta from PTU-induced hypothyroid rats produced consistently greater tension. This suggests that changes other than just those linked to altered adrenoreceptor activity occur in aortae from hyperthyroid and hypothyroid rats.

LITERATURE CITED

Altura, B. M.; Malaviza, D.; Reich, C. F.; Orkin, L. R. Effects of vasoactive agents on isolated human umbilical arteries and veins. Am. J. Physiol. 222(2): 345-355; 1972.

Banerjee, S. P.; Kung, L. S. B-adrenergic receptors in rat heart: Effects of thyroidectomy. Eur. J. Pharm. 43:207-208; 1977.

Bevan, J. A.; Osher, J. V. Effects of potassium on the resting length of vascular smooth muscle of the rabbit aorta and its response to I-norepinephrine. Circulation Research 13:346-351; 1963.

Biamino, G.; Wessel, H. J. Potassium induced relaxation of vascular smooth muscle: A possible mechanism of exercise hyperemia. Pfluegers Arch. Eur. J. Physiol. 343:95-106; 1973.

Bilezikian, J. P.; Loeb, J. N.; Gammon, D. E. The influence of hyperthyroidism and hypothyroidism on the B-adrenergic responsiveness of the turkey erythrocytes. J. Clin. Invest. 63:184-192; 1979.

Bohr, D. F.; Goulet, P. L. Role of electrolytes in the contractile machinery of vascular smooth muscle. Am. J. Cardiol. 8:549-556; 1961.

Bonaccorsi, A.; Heimsmeyer, K.; Smith, C. B.; Bohr, D. F. Norepinephrine release in isolated arteries induced by

K-free solution. Am. J. Physiol. 232(2):H140-H145; 1977a.

Bonaccorsi, A.; Heimsmeyer, K.; Aprigliano, O.; Smith, C. B.; Bohr, D. F. Mechanism of potassium relaxation of arterial muscle. Blood Vessels 14:261-276; 1977b.

Dillmann, W. H. Thyroid hormones and the heart. Thyroid Today 6(2):1-6; 1983.

Friedman, S. M.; Nakashiona, M.; Palaty, V.; Walters, B. K. Vascular resistance and Na^+-K^+ gradient in the perfused rat tail artery. Can. J. Physiol. Pharmacol. 51:410-417; 1973.

Ginsberg, A. M.; Clutter, W. E.; Shah, S. D.; Cryer, P. E. Tricodothyronine-induced thyrotoxicosis increases mononuclear leukocyte B-adrenergic receptor density in man. J. Clin. Invest. 67:1785-1791; 1981.

Gross, G.; Brodde, O. E.; Schumann, H. J. Decreased number of B-adrenoreceptors in cerebral cortex of hypothyroid rats. Eur. J. Pharmacol. 61:191-194, 1980.

Norton, J. M.; Detar, R. Potassium and isolated coronary vascular smooth muscle. Am. J. Physiol. 222(2):474-479; 1972.

Olszewski, M. C. The effect of PTU and thyroxine on blood vessels of young male Sprague-Dawley rats; Thesis; 1984.

Price, J. M.; Davis, D. L.; Knauss, E. B. Length-dependent sensitivity in vascular smooth muscle. Am. J. Physiol.

241:H557-H563; 1981.

Sparks, H. V. Effects of local metabolic factors on vascular smooth muscle. Handbook of physiology: The cardiovascular system. Vol. 2. Bethesda: American Physiological Society; 1980:253-299.

Stiles, G. L.; Lefkowitz, R. J. Hormone-sensitive adenylate cyclase. J. Biol. Chem. 257(11):6287-6291; 1982.

Stiles, G. L.; Stadel, J. M.; De Lean, A.; Lefkowitz, R. J. Hypothyroidism modulates beta adrenergic receptor-adenylate cyclase interactions in rat reticulocytes. J. Clin. Invest. 68:1450-1455; 1981.

Stratton, D. B. Contractility changes in thoracic aortae of young male thyroxine and propylthiouracil treated rats. Federation Proceedings 44(3):455; 1985.

Tsai, J. S.; Chen, A. Effect of L-triiodothyronine on (-)^{3}H-dihydroalprenolal binding and cyclic AMP response to (-) adrenaline in cultured heart cells. Nature 275:138-140; 1978.

Vanhoutte, P. M.; Lorenz, R. R. Inhibition of norepinephrine uptake by potassium ions in vascular smooth muscle. Artch. Intern. Pharmacodyn. Therap. 208:377; 1974.

Wahlstrom, B. A. A study on the action of noradrenaline on ionic content and sodium, potassium and chloride effluxes in the rat portal vein. Acta. Physiol. Scand.

89:522-530; 1973.

Waynforth, H. B. Thyroidectomy. Experimental and surgical technique in the rat. London: Academic Press; 1980:206-208.

Williams, L. T.; Lefkowitz, R. J.; Watanabe, A. M.; Hathaway, D. R.; Besch, H. R. Thyroid hormone regulation of B-adrenergic receptor number. J. Biol. Chem. 252(8):2787-2789; 1977.